

Claims:

- 5 1 A method for automatic alignment of tilt series (2-i) in an electron microscope, comprising:
- * applying markers (8-i) to a sample (2) to be imaged by the electron microscope;
 - * providing a tilt series of images (2-i) of the sample;
 - * identifying a first set of candidate markers (8-i) in each of the images in the tilt
- 10 series;
- * attributing at least one probability parameter to each candidate marker in each image;
- characterized in that the method further comprises:
- * selecting a second set (16-i) as a subset of candidate markers from the first set of
- 15 candidate markers on the basis of said at least one probability parameter;
- * projecting the candidate markers in the second set onto a sole image;
 - * applying a fitting algorithm to determine a set of parallel straight lines or very elongate ellipses (20-i) best fitting the candidate markers in the sole image;
 - * aligning the images in the tilt series on the basis of the identified candidate
- 20 markers.
- 2 2 A method according to Claim 1 in which the fitting algorithm used to determine the set of parallel straight lines comprises the Hough transformation.
- 25 3 3 A method according to Claim 1 in which the fitting algorithm used to determine the set of parallel straight lines or to determine a set of very elongate ellipses is constituted by the Generalized Hough transformation.

- 4 A method according to Claim 1 in which, before identifying candidate markers in each of the images in the tilt series, a cross correlation process is applied to the images of the tilt series.
- 5 A method according to any of the preceding Claims in which the probability parameter is derived from at least one of the quantities: size of the marker and local contrast of the marker.
- 6 A method according to Claim 2 in which the fitting algorithm further comprises:
- 10 * deriving for each candidate marker a sine-shaped curve (24) based on the co-ordinates of the corresponding candidate marker, according to the Hough transformation;
- * deriving from the sine-shaped curves a number of histograms (fig.4) indicating, for each direction (α), the relation between the density of candidate markers and the line
- 15 distance parameter (r) according to the Hough transformation;
- * applying an entropy (S) operation to each of the histograms, resulting in a set of entropy parameters (S_i), one entropy parameter for each histogram;
- * establishing the minimum value in the set of entropy parameters;
- * identifying the histogram corresponding to said minimum value as the one
- 20 showing the highest degree of peak diversity;
- * selecting from the latter histogram a number of peaks;
- * deriving from each peak position in the histogram the corresponding line distance parameter according to the Hough transformation.